



# **Decontamination of Biological Agents from the Surface of Materials of Military Importance**

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<b>Report Documentation Page</b>			Form Approved OMB No. 0704-0188	
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1. REPORT DATE <b>19 NOV 2003</b>	2. REPORT TYPE <b>N/A</b>	3. DATES COVERED <b>-</b>		
<b>4. TITLE AND SUBTITLE</b> <b>Decontamination of Biological Agents from the Surface Materials of Military Importance</b>			5a. CONTRACT NUMBER	
			5b. GRANT NUMBER	
			5c. PROGRAM ELEMENT NUMBER	
<b>6. AUTHOR(S)</b>			5d. PROJECT NUMBER	
			5e. TASK NUMBER	
			5f. WORK UNIT NUMBER	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> <b>Naval Surface Warfare Center Dahlgren Division Dahlgren, Virginia</b>			8. PERFORMING ORGANIZATION REPORT NUMBER	
<b>9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>			10. SPONSOR/MONITOR'S ACRONYM(S)	
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
<b>12. DISTRIBUTION/AVAILABILITY STATEMENT</b> <b>Approved for public release, distribution unlimited</b>				
<b>13. SUPPLEMENTARY NOTES</b> <b>See also ADM001851, Proceedings of the 2003 Joint Service Scientific Conference on Chemical &amp; Biological Defense Research, 17-20 November 2003. , The original document contains color images.</b>				
<b>14. ABSTRACT</b>				
<b>15. SUBJECT TERMS</b>				
<b>16. SECURITY CLASSIFICATION OF:</b>			<b>17. LIMITATION OF ABSTRACT</b> <b>UU</b>	
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>	18. NUMBER OF PAGES <b>19</b>	19a. NAME OF RESPONSIBLE PERSON



# *Outline*

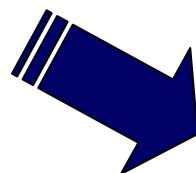
- Objectives
- Requirements & Guidelines
- Test Challenges
- Procedures
- Results & Conclusions
- Future Issues





# *Objectives*

- Develop method to determine the efficacy of candidate decontaminants on surfaces of military importance
- Begin to bridge the gap between laboratory testing and ‘real world’ application





# *Requirements & Guidelines*

- Utilize decontamination procedures in FM 3-5 as a guideline, modifying for the evaluation of decontaminants
- Provide quantitative assessment of decontaminant(s) efficacy





# Requirements & Guidelines

## Test Layout

- Surfaces
  - Sand, soil, concrete, asphalt
    - ~1 cm<sup>2</sup> surface area
    - Approximately 1 inch (2.54 cm) deep
- Challenge levels
  - *Bacillus anthracis* spores: 10<sup>6</sup> spores/cm<sup>2</sup>  
(JSFDS TEMP: Table 1-6)
- Replicates: Five
- Decontaminant amounts (FM 3-5)
  - Based on amount of decontaminant required for 10 g/m<sup>2</sup> chemical agent challenge (50:1 w/w)



# *Requirements & Guidelines*



## Test Layout (cont.)

- Allow contamination to remain 60 min prior to decontamination (NATO requirements AEP 7)
- Decontaminant contact time: 30 min. (FM 3-5)



## *Test Challenges*

- Agent application to material surface
- Decontaminant application to the surface
- Agent recoverability from the surface
- Distinguish between bacteriostatic and bacteriocidal effect
- Reduction of background contamination on surfaces
- Effective quench/neutralization of decontaminant after 30 minutes



# *Procedure*

- Select appropriate method to quench decontaminant
  - Example: For oxidizers, consider a reducing agent, such as sodium metabisulfite or sodium thiosulfate
  - Determine appropriate concentration of neutralization solution
    - Use an excess of neutralizer based upon molar ratios
    - Assay solution to verify absence of active component after neutralization
- Neutralize decontaminant and test on agent
  - Demonstrate minimal effect of neutralized decontaminant on viability of biological agent
  - Demonstrate recoverability of spores from surfaces



# Procedure

## Eliminate background contaminants from the surfaces

Spiked and untreated controls were tested



### *Soil & Sand*

- Autoclaved 60 minutes at 132°C , 28.5 psi, on a dry cycle
- Dried for 2 hours at 120 °C in dry convection oven



### *Asphalt & Concrete*

- Boiled for 5 minutes
- Dried at 120 °C in dry convection oven
  - Asphalt – 2 hours
  - Concrete – 1 hour

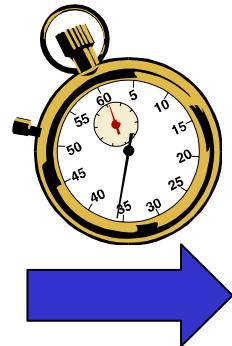


# Procedure

Add Agent



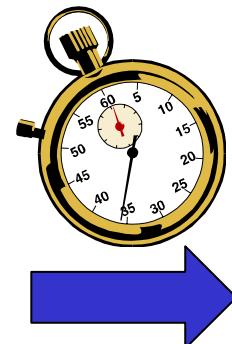
60 minutes



Add Decontaminant



30 minutes



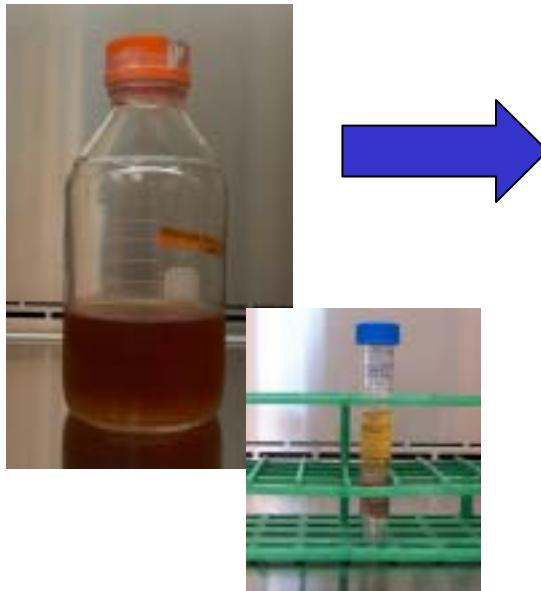
Neutralize



# Procedure

## Add Media

- Final volume = 5 ml



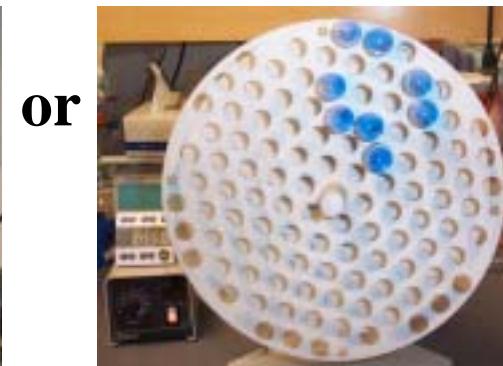
## Vortex

- Sand & soil



## Rotating Shaker

- Concrete & asphalt

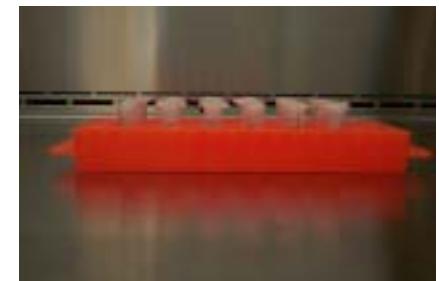


## Viability Assay



# Procedure

- Serial dilutions from each sample
  - Perform dilutions in media (900 µl media per tube)
- Plate 100 µl from each dilution tube, in duplicate
- Incubate at 37°C
  - Plates – 48 hours
  - Dilution tubes – up to 20 days (determined by test deadline)
  - Sample containing material - incubate with shaking

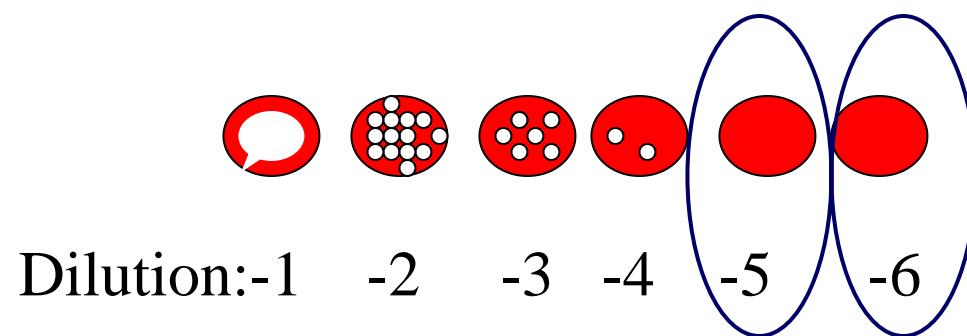




# Procedure

## Determine Efficacy of Decontaminant

- Count colony forming units
- Select highest two dilutions where zero growth is observed
  - Pipette remaining volume in each corresponding dilution tube into fresh 25 ml of media
  - Allow tubes to incubate in shaker/incubator for 48 hrs
  - Subsample tubes and plate in duplicate on appropriate media
  - Incubate plates and count colony forming units





# Results & Conclusions



- To date, we have tested this procedure on:
  - Peroxygen-based decontaminants
  - Alkaline
  - Acidic
  - Hypochlorite-based decontaminants
- Recoverability of biological agents from surfaces treated with *neutralized* decontaminant
  - Not significantly different from untreated surfaces ( $p<0.05$ )
  - Minimal variability within replicates ( $<0.5 \log$ )
- Decontaminant efficacy results were consistent (minimal variability) for plastic and stainless steel substrates
- Variable decontaminating results were observed ( $>2 \log$  difference) when asphalt, concrete, soil or sand surfaces were treated with *active* decontaminant



# *Future Issues*

- Porous surfaces (asphalt, concrete, soil, sand, etc.)
  - Interaction between biological agent and decontaminant
  - Interaction between decontaminant and surface materials
  - Aggregation of spores
- Application of agent and decontaminant
- Number of days to incubate dilution tubes
- Media selection
- Standardization of procedures
- Validation





# *Acknowledgements*



We thank the Joint Service Family of Decontamination Systems (JSFDS) Program for their support.

# *Backup Slides*



# References

## NATO References

Quadripartite Standardization Agreements (QSTAG),  
Standard 747, Edition 2, AEP-7. *NBC Survivability  
Acceptance Criteria, Design Guidelines, and Test  
Procedures for Defense Equipment Decontamination  
Survivability Criteria for Military Equipment*, Section II.  
Acceptance Criteria.

## FM 3-5 References

### Decontamination Stations

#### “Detailed Equipment Decon” section

- Pages 4-18 through 4-22
- Pages 4-19 through 4-23 (Change 1, 31 Jan 02)



## *References*

### **Calculation of Decontaminant Amount**

$$(10\text{g agent/m}^2) \times (50\text{g decon/g agent}) = 500\text{g decon/m}^2$$

$$(500\text{g decon/m}^2) \times (\text{m}^2/10^4 \text{ cm}^2) = 0.05\text{g decon/cm}^2$$